

Application No.: 10/517,741  
Attorney Docket No.: 47675-93  
First Applicant's Name: John Foekens  
Application Filing Date: 03 January 2006  
Office Action Dated: 24 November 2008  
Date of Response: 26 May 2009  
Examiner: Carla J. Myers

IN THE CLAIMS:

Applicants, pursuant to 37 C.F.R. § 1.121, submit the following amendments to the claims:

1. (Currently amended) A method for predicting the responsiveness of a human subject with a breast tissue cell proliferative disorder to a therapeutic treatment~~therapy~~, comprising:

obtaining, prior to or during therapeutic treatment of a subject having a breast tissue cell proliferative disorder, a biological sample comprising genomic DNA from the subject, wherein the therapeutic treatment comprises treatment with one of more drugs that target the estrogen receptor pathway or that are involved in estrogen metabolism, production or secretion; and

determining the genomic DNA methylation status of at least one CpG dinucleotide of at least one target nucleic acid sequence of the PITX2 gene, and the regulatory regions thereof selected from the group consisting of essentially SEQ ID NO:83, sequences complementary thereto, and contiguous portions thereof, by contacting the at least one target nucleic acid sequence with one or more agents suitable to convert cytosine bases that are unmethylated at the 5'-position thereof to a base that is detectably dissimilar to cytosine in terms of hybridisation properties, wherein hypomethylation is indicative for a low risk for relapse while hypermethylation is indicative for a high risk for relapse, wherein predicting responsiveness of the subject to the ~~therapy~~therapeutic treatment is afforded.

2.-19. (Cancelled)

20. (Currently amended) The method of claim 1, wherein said breast tissue cell proliferative disorder ~~of the breast tissue~~ is selected from the group consisting of ductal carcinoma in situ, lobular carcinoma, colloid carcinoma, tubular carcinoma, medullary carcinoma, metaplastic carcinoma, intraductal carcinoma in situ, lobular carcinoma in situ and papillary carcinoma in situ.

21. (Previously presented) The method of claim 1, wherein said subject is at least one of estrogen and progesterone receptor positive.

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22. (Currently amended) The method of claim 1, wherein said therapeutic treatmentmenttherapy is for the treatment of a relapse or metastatic cell proliferative disorder of the breast tissues.

23. (Currently amended) The method of claim 1, wherein said therapeutic treatmentmenttherapy is an adjuvant treatment.

24. (Previously presented) The method of claim 23, wherein said subject did not receive a chemotherapeutic treatment.

25.-44. (Cancelled)

45. (Currently amended) A method for predicting the responsiveness of a human subject with a breast cell proliferative disorder to a therapeutic treatmentmenttherapy, comprising:

obtaining, prior to or during therapeutic treatment of a subject having a breast tissue cell proliferative disorder, a biological sample comprising genomic DNA from the subject, wherein the therapeutic treatment comprises treatment with one of more drugs that target the estrogen receptor pathway or that are involved in estrogen metabolism, production or secretion;

isolating the genomic DNA;

contacting the isolated genomic DNA, or a portion thereof, with an agent or combination of agents suitable to convert cytosine bases that are unmethylated at the 5-position to uracil, or to another base which is dissimilar to cytosine in terms of base pairing behavior, to provide a pretreated DNA;

amplifying at least one pretreated DNA sequence, or a portion thereof, selected from the sequence group consisting of essentially SEQ ID NOS:411, 412, ~~515, 516~~, 685, 686, ~~789, 790~~, sequences complementary thereto, and contiguous portions thereof; and

determining, based on the amplification or on analysis of amplificates generated by the amplification ~~the nucleic acid amplificate~~, the methylation status of one or more genomic CpG dinucleotidedinucleotide sequences of the sequence ~~group consisting of~~ SEQ ID NO:83 ~~and SEQ ID NO:135~~, wherein hypomethylation is indicative for a low risk for relapse while hypermethylation is indicative for a high risk for relapse, wherein predicting responsiveness of the

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subject to the ~~therapy~~therapeutic treatment is afforded.

46.-56. (Cancelled)

57. (Previously presented) The method of claim 45, wherein determining the methylation status comprises sequencing.

58. (Previously presented) The method of claim 45, wherein amplifying comprises using methylation-specific primers.

59. (Currently amended) The method of claim 45, wherein amplifying comprises use of at least one nucleic acid molecule or peptide nucleic acid molecule comprising in each case a contiguous sequence at least 9 nucleotides in length that is complementary to, or hybridizes under moderately stringent or stringent conditions to a sequence selected from the group consisting of essentially SEQ ID NOS:411, 412, ~~515, 516~~, 685, 686, ~~789, 790~~, sequences complementary thereto, and contiguous portions thereof, wherein said at least one nucleic acid molecule or peptide nucleic acid molecule suppresses amplification of a nucleic acid to which it is hybridized.

60. (Cancelled)

61. (Previously presented) The method of claim 45, wherein contacting is with an agent, or combination of agents, comprising at least one of bisulfite, hydrogen sulfite or disulfite.

62. (Currently amended) A method for predicting the responsiveness of a subject with a breast cell proliferative disorder to a therapeutic treatment~~therapy~~, comprising:

obtaining, prior to or during therapeutic treatment of a subject having a breast tissue cell proliferative disorder, a biological sample comprising genomic DNA from the subject, wherein the therapeutic treatment comprises treatment with one of more drugs that target the estrogen receptor pathway or that are involved in estrogen metabolism, production or secretion;

isolating the genomic DNA;

digesting the isolated genomic DNA, or a portion thereof, comprising at least one sequence selected from the sequence group consisting of essentially SEQ ID NO:83, ~~SEQ ID NO:135~~, sequences complementary thereto, and contiguous portions thereof, with one or more methylation-

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sensitive restriction enzymes; and

determining the DNA fragments generated or not generated, wherein the methylation status of a least one CpG dinucleotide of said at least one sequence is determined, wherein hypomethylation is indicative for a low risk of relapse while hypermethylation is indicative for a high risk of relapse, and wherein predicting responsiveness of the subject to the therapy is afforded.

63.-66. (Cancelled)

67. (Currently amended) The method of claim 62, further comprising, prior to determining the DNA fragments, amplifying the ~~DNA digest~~ DNA fragments generated.

68.-76. (Cancelled)

77. (Previously presented) The method of any one of claims 45 and 62, wherein the biological sample containing genomic DNA is obtained from a source selected from the group consisting of cells or cellular components which contain DNA, cell lines, histological slides, biopsies, tissue embedded in paraffin, breast tissues, blood, plasma, lymphatic fluid, lymphatic tissue, duct cells, ductal lavage fluid, nipple aspiration fluid, bone marrow, and combinations thereof.

78.-80. (Cancelled)